Prevalence and molecular characterization of C. pecorum detected in Swiss fattening pigs

Rohner, Lea ; Marti, Hanna ; Torgerson, Paul ; Hoffmann, Karolin ; Jelocnik, Martina ; Borel, Nicole

Abstract: Chlamydia (C.) pecorum, an obligate intracellular bacterial species commonly found in ruminants, can also occur in pigs. However, its significance as a potential porcine pathogen, or commensal, is still unclear. In a previous study (Hoffmann et al. 2015), mixed infections of C. suis and C. pecorum were detected in 14 Swiss fattening pig farms. Using these samples, we aimed to investigate the infection dynamics of C. suis and C. pecorum mixed infections in these farms. In addition, we analyzed the genetic diversity of Swiss porcine C. pecorum strains in relation to globally circulating strains. In total, 1284 conjunctival and rectal swabs from 391 pigs, collected at the beginning and end of the fattening period, were tested during the course of this study. We determined the bacterial loads of C. suis and C. pecorum using species-specific real-time PCR (qPCR) and compared these results to already existing DNA-microarray and Chlamydiaceae qPCR data. Overall, C. suis and Chlamydiaceae copy numbers decreased in the course of the fattening period, whereas C. pecorum copy numbers increased. No association was found between clinical signs (conjunctivitis, lameness and diarrhea) and the bacterial loads. Preventive antibiotic treatment at the beginning of the fattening period significantly lowered the chlamydial load and outdoor access was associated with higher loads. Proximity to the nearest ruminants correlated with increased C. pecorum loads, indicating that C. pecorum could be transmitted from ruminants to pigs. Multi-locus sequence typing (MLST) and major outer membrane protein (ompA) genotyping revealed two novel sequence types (STs) (301, 302) and seven unique ompA genotypes (1-7) that appear to form a specific clade separate from other European C. pecorum strains

DOI: https://doi.org/10.1016/j.vetmic.2021.109062

Prevalence and molecular characterization of *C. pecorum* detected in Swiss fattening pigs

Lea Rohner [a,b^], Hanna Marti [a,b^*], Paul Torgerson [c], Karolin Hoffmann [a,b], Martina Jelocnik [d], and Nicole Borel [a,b]

a. Institute of Veterinary Pathology, Vetsuisse-Faculty University Zurich, Zurich, 8057, Switzerland
b. Center for Clinical Studies, Vetsuisse-Faculty, University of Zurich, Zurich, 8057, Switzerland
c. Section of Veterinary Epidemiology, Vetsuisse-Faculty, University of Zurich, Zurich, 8057, Switzerland
d. Genecology Research Centre, University of the Sunshine Coast, Sippy Downs, 4556, Queensland, Australia

^ depicts equal co-authorship

* Correspondence:

Dr. med. vet., Dr. sc. nat. Hanna Marti
Institute of Veterinary Pathology
Vetsuisse-Faculty
University of Zurich
8057 Zurich
Switzerland
Email: hanna.marti@uzh.ch

Abstract

Chlamydia (C.) pecorum, an obligate intracellular bacterial species commonly found in ruminants, can also occur in pigs. However, its significance as a potential porcine pathogen, or commensal, is still unclear. In a previous study (Hoffmann et al. 2015), mixed infections of *C. suis* and *C. pecorum* were detected in 14 Swiss fattening pig farms. Using these samples, we aimed to investigate the infection dynamics of *C. suis* and *C. pecorum* mixed infections in these farms. In addition, we analyzed the genetic diversity of Swiss porcine *C. pecorum* strains in relation to globally circulating strains. In total, 1284 conjunctival and rectal swabs from 391 pigs, collected at the beginning and end of the fattening period, were tested during the course of this study. We determined the bacterial loads of *C. suis* and *C. pecorum* using species-specific real-time PCR (qPCR) and compared these results to already existing DNA-microarray and *Chlamydiaceae* qPCR data. Overall, *C. suis* and *Chlamydiaceae* copy numbers decreased in the course of the fattening period, whereas *C. pecorum* copy numbers increased. No association was found between clinical signs (conjunctivitis, lameness and diarrhea) and the bacterial loads. Preventive antibiotic treatment at the beginning of the fattening period significantly lowered the chlamydial load and outdoor access was associated with higher loads. Proximity to the nearest ruminants correlated with increased *C. pecorum* loads, indicating that *C. pecorum* could be transmitted from ruminants to pigs. Multi-locus sequence typing (MLST) and major outer membrane protein (*ompA*) genotyping revealed two novel sequence types (STs) (301, 302) and seven unique *ompA* genotypes (1-7) that appear to form a specific clade separate from other European *C. pecorum* strains.

Keywords

porcine *Chlamydia pecorum;* MLST; genotyping; fattening pigs; bacterial load; real-time PCR; quantitative PCR

Introduction

The gram-negative, obligate intracellular *Chlamydiaceae* family comprises one genus *Chlamydia* (*C.*) consisting of fourteen described species to date. The important livestock pathogen *C. pecorum* can infect a variety of different host species including cattle and pigs, but it is also responsible for severe ocular disease, urinary tract infections and infertility in koalas (Sachse and Borel, 2020). While its clinical impact on pigs has yet to be fully elucidated (Schautteet and Vanrompay, 2011), *C. pecorum* has been associated with keratoconjunctivitis, polyarthritis, encephalomyelitis and reduced livestock productivity in ruminants (Sachse and Borel, 2020).

In contrast, *C. suis* primarily infects the intestinal tract of pigs without causing any or only mild clinical signs such as diarrhea (Schautteet and Vanrompay, 2011; Hoffmann et al., 2015). Despite its minor impact on porcine health, *C. suis* has become a major concern in recent years because it is the first obligate intracellular bacterium known to have naturally acquired stable antibiotic resistance by integrating a tetracycline resistance gene into its chromosome (Dugan et al., 2004). Furthermore, veterinarians, pig farmers and slaughterhouse workers are at risk of contracting *C. suis* (De Puysseleyr et al., 2014, 2017), and natural co-infections of *C. suis* and *C. trachomatis* have been detected in the eyes of trachoma patients in Nepal and Sudan (Dean et al., 2013; Ghasemian et al., 2018). Thus, natural transmission of tetracycline resistance from *C. suis* to *C. trachomatis* cannot be excluded (Joseph et al., 2016).

In a previous study, we investigated the prevalence of *Chlamydia* in Swiss fattening pigs. Rectal and conjunctival swabs were collected from 636 fattening pigs out of 29 different farms at the start and end of the fattening period (Hoffmann et al., 2015). While *C. suis* was found on all farms, *C. pecorum* was primarily detected at the end of the fattening period in 48% of all farms (n=14). All *C. pecorum*-positive animals were also positive for *C. suis*, which is indicative for co-infection.

Little is known about the clinical impact and distribution of *C. pecorum* in pigs because studies on *Chlamydiaceae* in pigs have primarily focused on *C. suis* (Schautteet and Vanrompay, 2011; Sachse and Borel, 2020). Moreover, only few studies have performed phylogenetic analyses of porcine *C. pecorum* strains (Kaltenboeck and Storz, 1992; Mohamad et al., 2014; Jelocnik et al., 2015a). Due to its recombinogenic nature, the historically often sequenced major outer membrane protein (*ompA*) is no longer recommended for molecular typing without additional multi-locus sequence typing (MLST) or the use of related methods (Pannekoek et al., 2008; Pillonel et al., 2019). However, *ompA* genotyping is still common for phylogenetic analyses of the *Chlamydiaceae* family because the locus is highly variable and there is a large database available for comparative analysis (Li et al., 2017).

In this study, we aimed to investigate the infection dynamics of *C. suis* and *C. pecorum* in fattening pig farms and to perform molecular typing of *C. pecorum* strains from 14 *C. pecorum*-positive farms (Hoffmann et al., 2015).

Material and Methods

Sample collection and workflow

In a previous study (Hoffmann et al., 2015), conjunctival and rectal swabs (FLOQSwabs, Copan Flock Technologies, Brescia, Italy) were collected from fattening pigs of 29 different farms between September 2013 and December 2014. The farms were visited twice, once at the beginning of the fattening period (sampling timepoint a) and three months later before slaughter (timepoint b). The Veterinary Office of Canton Luzern approved the study (authorization no. LU03/14) and all efforts were made to minimize the discomfort of the animals during sampling. The owners of the pig housings and properties, from where the samples were collected, gave their permission to conduct the study on these sites. In 14 of these farms (Hoffmann et al. farms no. 4, 6, 9, 11, 14, 15, 16, 10, 20, 22, 24-26, 28; referred to as “HK farms” throughout the text),

mixed infections with *C. suis* and *C. pecorum* were diagnosed by the Arraymate microarray technology (described in detail below). In this study, we re-analyzed mixed infected samples using species-specific qPCRs to determine the bacterial load of each chlamydial species individually (Figure 1). Altogether, we selected 1184 DNA samples (592 conjunctival and 592 rectal swabs) from 341 pigs of the previous study (Hoffmann et al., 2015). Furthermore, we extracted DNA from 100 additional samples of the selected 14 farms to increase the number of potentially mixed infected samples (HK farms 4, 6, 14, 20 and 26).

DNA was extracted from all new samples followed by a *Chlamydiaceae* family-specific qPCR. Positive samples were then further analyzed with the Arraymate microarray, and bacterial loads were quantified using the *C. suis* and the *C. pecorum*-specific qPCRs (Figure 1). Detailed protocols are listed below.

DNA extraction and quality control

DNA was extracted as described (Hoffmann et al., 2015) using the Maxwell 16 Buccal Swab LEV DNA Purification Kit (Promega, Madison, WI, USA) adhering to instructions provided by the manufacturer. All swabs were eluted in 100 µl elution buffer.

DNA concentration and quality were determined using Nanodrop-1000 (Witec AG, Lucerne, Switzerland). Samples with DNA concentrations above 120 ng/µl were marked and diluted 1:10 prior to analysis with *Chlamydiaceae*, *C. suis* and *C. pecorum* qPCRs.

Chlamydiaceae-specific qPCR screening and species identification with Arraymate microarray

Initial screening for *Chlamydiaceae* and species identification with Arraymate microarray were performed as described (Hoffmann et al., 2015). Specifically, the *Chlamydiaceae* family-

specific qPCR targets a 111 bp segment of the 23S ribosomal RNA (rRNA) (Ehricht et al., 2006), and was performed on an ABI 7500 instrument (Applied Biosystems, Foster City, CA, USA). An internal amplification control (117 bp) was included using Intype IC-DNA (Hoffmann et al., 2015). All samples were tested in duplicate with a cycle threshold value of 0.1 set after each run. A cycle threshold (Ct value) < 38 was considered positive. A *C. abortus* standard curve was used for quantification and a water sample served as a negative control. If the internal amplification control was inhibited, the sample was tested again with a 1:10 dilution.

Next, all positive samples were investigated with the Arraymate microarray, which targets a multivariable sequence on the 23S rRNA gene (Alere, Jena, Germany) allowing identification of twelve *Chlamydiaceae* species: *C. abortus, C. avium, C. caviae, C. felis, C. gallinaceae, C. ibidis, C. muridarum, C. pecorum, C. pneumoniae, C. psittaci, C. suis* and *C. trachomatis,* as well as the identification of mixed infections (Schnee and Sachse, 2015). This array is based on hybridization of DNA, which is first amplified and biotin-labeled by PCR with the following thermocycler protocol: 96°C 10 min, 40 cycles of 94°C 30 s, 50°C 30 s and 72°C 30 s. Internal control DNA was included, as recommended by the manufacturer (Intype IC-DNA, Qiagen Labor, Leipzig) (Hoffmann et al., 2015). The microarray chip was then loaded with 8 µl of the amplified PCR product unless Ct-values were low (< 30, 4 µl). The *Chlamydia* species could not be determined if only genus-specific probes were positive or if species-specific probes produced no or only a weak signal. All primers and detailed thermocycler protocols used in this study are listed in Supplementary Data 1.

Species-specific qPCRs

Species-specific qPCRs target a 118 bp fragment of the 23S rRNA and a 76 bp fragment of the *ompA* gene specific for *C. suis* and *C. pecorum*, respectively. A sample was considered positive at Ct-values < 38 (*C. suis* qPCR) or < 40.5 (*C. pecorum* qPCR). Both qPCRs were published

previously (Pantchev et al., 2010) and all primers/probes, mastermixes and thermocycler protocols are listed in Supplementary Data 1.

For bacterial load quantification, standard curves were prepared using recombinant plasmids according to established methods (Ehricht et al., 2006). In detail, the species-specific DNA fragments were amplified using the *C. suis* type strain S45 (Kaltenboeck and Storz, 1992) and the porcine *C. pecorum* strain 1710S (Kaltenboeck and Storz, 1992) as templates. These fragments were then cloned separately into TOPO TA vectors with the TOPO TA Cloning Kit (Invitrogen, ThermoFisher Scientific, Carlsbad, CA, USA) according to manufacturer's instructions. Next, recombinant plasmids were purified with the GeneJET Plasmid Miniprep kit (ThermoFisher Scientific). DNA concentrations were measured with the Qubit fluorometric quantification method (Qubit 4 fluorometer, ThermoFisher Scientific). The number of copies / µl was then calculated with the following formula (1):

Copies / µl = (6.022E+23 molecules/mole x DNA concentration (g/µl)/[(No. of base pairs)*660 Da] (1)

Bacterial load determination

Standard curves ranged from 10 to 10^7 and 1 to 10^7 copies for the *Chlamydiaceae* and the species-specific qPCRs, respectively. The average number of copies per sample and qPCR was determined using the standard formula (1):

$Y = Ae^{bx}$ (1)

A = y-intercept, *e* = mathematical constant (2.71828), b = exponential growth constant

Ct-values (y) and corresponding copy numbers (x) of the standard curves were used to calculate A and b for each run. Two Ct-values per sample, qPCR and run were then used to calculate the average number of copies per swab.

Statistics

R was used for statistical analysis (https://www.r-project.org/). A series of generalized linear mixed models were used in the analysis using the glmmTMB and MCMCglmm packages (Hadfield, 2010; Brooks et al., 2017). Animal ID was used as random intercept whilst timepoint of sampling was a random slope. The chlamydial load was defined as the dependent variable and a generalized linear mixed model with a compound poisson-gamma (tweedie) distribution was used for analysis because the data was not distributed normally. A Q-Q plot was used on simulated residuals to verify this model as appropriate for the present data set.

For binominal clinical data such as conjunctivitis (yes/no), a binominal model was used with the clinical signs set as dependent variable, while the eye score (0-3) was analyzed with an ordinal model. A multivariable model was used to look for disruptive factors with sampling timepoint and type set as fixed variables.

C. pecorum-specific ompA genotyping and multi-locus sequence typing (MLST)

For molecular typing of different *C. pecorum* strains, we performed both *ompA* genotyping and *C. pecorum* specific-MLST.

For *ompA* genotyping, full-length primers were designed that cover 1201 bp of the *ompA* gene. For MLST, which targets the seven housekeeping genes *gatA, oppA_3, hflX, gidA, enoA, hemN* and *fumC*, established *C. pecorum*-specific primers were used (Jelocnik et al., 2019). All eight PCRs were prepared as 50 µl reactions comprising 1x AmpliTaq Gold 360 mastermix (ThermoFisher Scientific), 0.3 µM of each forward and reverse primer (Microsynth, Balgach,

198 Switzerland) and 3 µl DNA template. A negative control (*C. suis* S45 DNA) and a positive
199 control (*C. pecorum* W73) were included for each assay. Cycling conditions consisted of
200 10 min initialization at 95 °C, 40 cycles of 60 s denaturation (95 °C), 60 s primer-specific
201 annealing and 90 s elongation (72 °C), and final elongation for 7 min (72 °C). Primer
202 sequences, annealing temperatures and expected amplicon sizes are listed in Supplementary
203 Data 1 (Table S4). Next, PCR products, purified with the GeneJET PCR purification kit
204 (ThermoFisher Scientific), were sent for Sanger sequencing at Microsynth (Balgach,
205 Switzerland) using the forward and reverse primers as sequencing primers.
206 The Geneious Prime (v. 2020.2.4) software was used to *de novo* assemble the
207 chromatograms and extract consensus sequences from *ompA* and MLST gene fragments,
208 followed by additional sequence and phylogenetic analyses.
209 The *omp*A sequences from this study were aligned using ClustalOmega (as implemented
210 in Geneious Prime) to other publicly available *C. pecorum omp*A sequences retrieved from
211 GenBank (https://www.ncbi.nlm.nih.gov/genbank/). Using the 962 bp *omp*A alignment of the
212 49 *C. pecorum* strains described in this study and additional 35 previously published strains
213 from livestock (pig, cattle, sheep and goat) and koala hosts, we constructed a mid-point rooted
214 approximately-maximum-likelihood phylogenetic tree with Fasttree 2.1.11 using GTR
215 nucleotide substitution model (as implemented in Geneious Prime). The *omp*A sequences were
216 submitted to the NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/) under the accession
217 numbers MW132547-132595 (Supplementary Data 2).
218 For MLST, the resulting consensus sequences were trimmed to the appropriate length
219 (Jelocnik et al., 2019). Next, the seven fragments were concatenated in the order as they appear
220 on the genome (Pannekoek et al., 2008), *gatA, oppA_3, hflX, gidA, enoA, hemN* and *fumC,*
221 which resulted in 3095 bp long sequences (Jelocnik et al., 2013). The novel alleles and STs
222 (n = 18) were denoted using the *Chlamydiales* MLST database

(http://pubmlst.org/chlamydiales/). The concatenated sequences from this study were then aligned using ClustalOmega (as implemented in Geneious) to other publicly available livestock and koala *C. pecorum* MLST sequences (n = 33) retrieved from the Chlamydiales PubMLST database (https://pubmlst.org/organisms/chlamydiales-spp). Using this alignment, we also constructed a mid-point rooted approximately-maximum-likelihood phylogenetic tree with FastTree 2.1.11 using GTR nucleotide substitution model (as implemented in Genious Prime). The novel STs and alleles identified in this study are available in the Chlamydiales PubMLST database.

Additionally, a mid-point rooted approximately-maximum-likelihood phylogenetic tree was constructed using *omp*A and MLST alignments of a) 18 samples from this study, and b) 28 additional, published global *C. pecorum* sequences. The matching taxa on *omp*A tree were connected using auxiliary lines on the opposite MLST tree to create a tanglegram.

Results

There is no correlation between clinical signs and bacterial loads regardless of the chlamydial species

The previous study investigating 29 fattening pig farms found a correlation between diarrhea and fecal swabs positive for *Chlamydiaceae* but not between conjunctivitis and conjunctival swab positivity (Hoffmann et al., 2015). Here, we correlated the positivity and bacterial loads of *C. suis* and *C. pecorum* to the occurrence of clinical signs individually. For the 341 previously analyzed pigs, clinical data was available (Supplementary Data 3) (Hoffmann et al., 2015). Specifically, lameness and conjunctivitis status were assessed on farm level, whereas for each individual pig, the occurrence of diarrhea (yes/no) and conjunctivitis (yes/no) was noted (Table 1), with additional scoring of conjunctivitis signs (eye score 0-3) (Supplementary Data 3).

Across all 14 farms, 50/333 (15%) and 145/379 (38.3%) pigs showed signs of conjunctivitis at the beginning and end of the fattening period, respectively. Fewer animals displayed signs of diarrhea with 15 (4.5%) at the beginning and six (1.6%) at the end of the fattening period (Table 1). Lameness was determined on farm-level (Hoffmann et al., 2015), and 8/14 farms reported problems with lameness,.

For statistical analysis, a binominal model and an ordinal model were used for diarrhea/conjunctivitis and the conjunctivitis score, respectively, each model defining the clinical signs as dependent variables, and sampling site (eye/rectum), timepoint (a/b) and the individual animal as random variables. We found no association between high *Chlamydiaceae*, *C. suis* and *C. pecorum* loads and diarrhea, lameness, or conjunctivitis. Animals were significantly more likely to show signs of conjunctivitis and higher eye scores at the end of the fattening period compared to the beginning of the fattening period ($p < 2x10^{-16}$).

Prophylactic antibiotic treatment affects C. suis but not C. pecorum loads

Next, we investigated the influence of prophylactic medication on chlamydial positivity and chlamydial loads (n = 5 farms, Table 1). Conjunctivitis/eye scores were significantly lower if prophylactic antibiotic treatment was used regardless of the specific drug combination ($p < 2x10^{-16}$). The total *Chlamydiaceae (*$p = 0.044$*)* and specific *C. suis* ($p = 2.65x10^{-6}$) bacterial loads significantly increased from the first to the second sampling if no prophylactic antibiotics were applied on farm level. In contrast, *C. pecorum* bacterial loads were not affected by preventive antibiotic treatment.

C. suis loads decrease during the fattening period whereas C. pecorum loads increase

We then compared positivity rates as well as overall bacterial load dynamics of total *Chlamydiaceae*, *C. suis* and *C. pecorum* at the beginning and end of the fattening period

regardless of treatment. For the 14 farms analyzed in this study, the prevalence was 98.7% with only five animals yielding negative *Chlamydiaceae* qPCR results in all samples tested, specifically four animals from HK farm 22 and one animal from HK farm 26 (Supplementary Data 4, Table S1). *C. suis* prevalence as determined by species-specific qPCR was very similar to the *Chlamydiaceae* results (Supplementary Data 4, Table S2). In detail, all animals investigated with the *C. suis* qPCR tested positive in at least one sample except for animal no. 22 of farm 22 (22-22), where only a conjunctival but no rectal swab was available at the first sampling timepoint (Supplementary Data 3).

As previously described in the Hoffmann study (Hoffmann et al., 2015), the positivity rate of *C. pecorum* was generally lower than *C. suis*, notably increasing between the first and second sampling timepoint. Specifically, of the 290 animals investigated in this study, 181 pigs tested positive for *C. pecorum* in at least one of the four samples resulting in a prevalence of 62.4% (Table 2). For HK farm 22, the presence of *C. pecorum* could not be confirmed because no animal tested positive in the species-specific PCR. Across all 14 farms, the average chlamydial load found in rectal swabs was significantly higher compared to conjunctival swabs for all three qPCRs used in this study ($p < 2x10^{-16}$).

Chlamydiaceae and *C. suis* bacterial loads significantly decreased during the course of the fattening period, whereas *C. pecorum* loads increased from the first to the second sampling timepoint (Table 3), corresponding to the positivity rate dynamics as shown above (Table 2). Similar dynamics were observed if only rectal swabs were taken into consideration. In contrast, bacterial loads in the conjunctival swabs significantly increased from the first to the second sampling timepoint.

Close contact to ruminants correlates with increased C. pecorum but not C. suis loads at the second sampling timepoint

In the Hoffmann study, different risk factors were analyzed including access to free-range areas (outdoor access), contact to wild boars and ruminants as well as the animal husbandry system (Hoffmann et al., 2015). Here, we focused on risk factors that correlated with *C. pecorum* positivity, such as outdoor access and direct or indirect contact to ruminants (Hoffmann et al., 2015). We compared *Chlamydiaceae, C. suis* and *C. pecorum* bacterial loads with the occurrence of these risk factors (Supplementary Data 5). In total, only HK farm 25 had no outdoor access and six farms (6, 15, 16, 20, 22, 26) had direct contact to ruminants (i.e. cattle, sheep, goats).

Statistical analysis confirmed that animals with outdoor access yielded significantly higher bacterial loads of *Chlamydiaceae* ($p = 0.0141$) and *C. pecorum* ($p = 0.0122$), but not *C. suis,* compared to animals without access to free-range areas. Furthermore, we demonstrated that close proximity to ruminants resulted in significantly higher bacterial loads of *Chlamydiaceae, C. suis* and *C. pecorum* with p-values of 0.0018, 1.8×10^{-5} and 0.01, respectively.

Seven C. pecorum ompA genotypes and two MLST sequence types circulate in twelve fattening pig farms

The genomes of 29 *C. suis* isolates from nine out of the 29 HK farms (farms 1, 17, 22, 23, 25-29) have already been sequenced and compared to each other as well as put into a global context (Seth-Smith et al., 2017). In contrast, the genetic makeup of *C. pecorum* detected in this study is unknown. In order to close this knowledge gap, we determined *C. pecorum ompA* genotypes and MLST sequence types (ST) in a selection of samples from 12 out of 14 farms. For HK farm 25, *ompA* genotyping was attempted on ten different samples, but remained unsuccessful (data not shown). HK farm 22 was negative for *C. pecorum* in the species-specific qPCR and was therefore not included. In total, *ompA* genotyping was successfully performed on 49

samples resulting in seven different genotypes (denoted 1 to 7) with 84.3 to 100% sequence similarity (Supplementary Data 2).

Genotype 1 was the most commonly (n = 30) and widely distributed *ompA* genotype appearing on nine farms (HK farm 4, 6, 11, 14, 15, 20, 24 and 26) followed by genotype 5 (n = 9) from HK farms 6, 26 and 28. Genotypes 2 and 3 were each found in three samples from HK farms 9 and 19, respectively, while genotype 4 was detected in one sample each from HK farms 9 and 20 (17-9bR, 18-20bR). Finally, genotypes 6 (n = 1) and 7 (n = 1) were only detected on HK farm 16 (Supplementary Data 2).

As a next step, MLST STs were determined in a total of 18 samples from all twelve farms. MLST resulted in two novel STs, 301 and 302, detected on nine (HK farms 4, 9, 11, 14, 15, 19, 20, 24, 26) and three (HK farms 6, 16, 28) farms, respectively (Supplementary Data 2). Combining the seven *ompA* genotypes 1-7 with the two STs 301 and 302, we identified seven novel genotypes, arbitrarily denoted A to G, in 18 samples originating from twelve farms (Figure 2, Table 4). The phylogenetic analyses of *ompA* genotypes 1-7 and the STs 301 and 302 revealed two matched clades, one formed by genotypes A to D (*ompA* 1-4 + ST 301) and the other by genotypes E to G (*ompA* 5-7 + ST 302) (Figure 2).

While the seven novel porcine ompA genotypes 1-7 resolve into separate clades, MLST STs 301 and 302 cluster closely together compared to global C. pecorum strains retrieved from various host species

Finally, we aimed to put our data into a global context by comparing the seven novel *ompA* genotypes and the two STs to globally available *C. pecorum ompA* genotypes and STs from cattle (blue), sheep (orange), pig (red), goat (green), koala (purple), and deer/ibex (brown) hosts (Figure 3, Supplementary Data 6).

For *ompA* genotypes, genotypes 1-4 formed a distinct sub-clade, which includes a *C. pecorum ompA* sequence from Chinese cattle (strain F2137), within a larger genetically diverse clade that comprises *omp*A sequences from Australian koala (Marsbar), Italian cattle (5184) and Moroccan goat (M14). Genotypes 5-7 also formed their own well-supported clade within a larger second clade including *omp*A sequences from sheep strains originating from France, Ireland and the USA (iB5, W73 and 757, respectively, Figure 3A). In contrast, the STs described in this study formed a well-supported distinct clade separate from all other *C. pecorum* STs with the exception of ST 201 obtained from the feces of Australian cattle (Faecal_11) (Figure 3B). Both, *omp*A and MLST, phylogenetic trees as well the *omp*A/ST tanglegram demonstrate that Swiss porcine strains form distinct clades within the global *C. pecorum* population (Figure 3; Supplementary Data 7).

Discussion

While *C. suis* in pigs has been associated with mild clinical signs mostly affecting the respiratory and reproductive tract as well as causing conjunctivitis (Sachse and Borel, 2020), it is mostly present as a gastrointestinal commensal (Schautteet and Vanrompay, 2011). In contrast, much less is known about the occurrence of *C. pecorum* in pigs and its impact on porcine health (Schautteet and Vanrompay, 2011). In this study, we expanded previous analyses (Hoffmann et al., 2015) by focusing on the 14 farms known to harbor infections with *C. pecorum* and correlated the clinical data with positivity by qPCRs detecting *Chlamydiaceae*, *C. suis* and *C. pecorum*.

We found no correlation between any of the clinical signs and the positivity rates of either qPCR, suggesting that neither *C. suis* nor *C. pecorum* are primary pathogens in the investigated cohort. However, recurrent and/or chronic infection with *Chlamydia* can diminish the productivity of livestock on herd-level (Reinhold et al., 2011). Therefore, it cannot be excluded

371 that the high *Chlamydiaceae* burden in all farms, and especially in farms with *C. suis* and
372 *C. pecorum* co-infections, could have had an impact on herd health .

373 Only few studies have so far investigated the correlation between clinical signs and the
374 chlamydial burden. One study found that higher bacterial loads of *C. suis* (Ct-value < 36) were
375 more likely to correlate with conjunctivitis than lower bacterial loads (Ct < 38) (Englund et al.,
376 2012) and experimental studies demonstrated that high infectious doses had an impact on the
377 severity of disease (Guscetti et al., 2009). However, there has been no in-depth study
378 comparing the chlamydial loads of pigs from farms with *Chlamydia*-related problems such as
379 conjunctivitis (Schautteet et al., 2010; Unterweger et al., 2020) with farms without (Hoffmann
380 et al., 2015) in order to confirm the interplay between bacterial load and clinical signs.

381 Furthermore, we found that the *Chlamydiaceae/C. suis* burden significantly decreased
382 between sampling timepoints, especially if antibiotic prophylaxis was applied. A possible
383 explanation for this finding could be immune-modulating effects induced by preventive
384 antibiotic treatment (Jensen et al., 2014). These immune-modulating effects might have also
385 contributed to the lower eye scores in treated animals at the end compared to the start of the
386 fattening period. Preventive antibiotic treatment was administered orally for five to twelve days
387 at the beginning of the fattening period, shortly after the first sampling timepoint (Hoffmann
388 et al., 2015). *C. pecorum* load and positivity remained unaffected by antibiotic prophylaxis
389 indicating that infection occurred after treatment and thus originated from an outside source,
390 most likely from ruminants.

391 In general, the overall bacterial load and the prevalence of *C. suis* were considerably higher
392 than those of *C. pecorum* confirming previous observations that *C. suis* is the primary
393 chlamydial species detected in pigs, although single and mixed infections with other species
394 are possible (Schautteet and Vanrompay, 2011). Interestingly, while three pigs from HK
395 farm 22 tested *C. pecorum*-positive by DNA-microarray at the end of the fattening period

(Hoffmann et al., 2015), this finding could not be confirmed by *C. pecorum*-specific qPCR. One explanation for these differing results could be that initial *C. pecorum* loads of these samples were already low, and that the sample quality further degraded over time.

This study focused on *C. pecorum* infections and related risk factors, which were already identified as 'outdoor access' and 'contact to ruminants' (Hoffmann et al., 2015). In addition to confirming previous findings, we found that increasing proximity to ruminants significantly increased bacterial loads of *C. pecorum* but also of *C. suis,* and *Chlamydiaceae* in general. Taken together, we conclude that domestic ruminants are a possible source of chlamydial infection for pigs. Infection likely occurs via the fecal-oral route (Li et al., 2016), and to a lesser extent via abortion material (Giannitti et al., 2016). Outdoor access as a risk factor could directly correlate with contact to ruminants, but could also result from contact to wild boars and wild ruminants. However, considering the low prevalence of *Chlamydia* in the Swiss wild boar (Wahdan et al., 2020) and wild ruminant population (Holzwarth et al., 2011), the latter appears to be unlikely, even though these studies were not performed in the same geographical regions. One major limitation to analyses concerning outdoor access is that only one farm (HK farm 25) provided no outdoor access.

We then genotyped porcine *C. pecorum* strains to investigate the relationship between our strains and *C. pecorum* strains from domestic and wild ruminants as well as koalas and pigs from other countries. On the investigated farms, we detected seven distinct *ompA* genotypes. When compared to the globally distributed strains from various hosts, genotypes 1-4 and 5-7 clustered into two genetically distinct separate sub-clades. Interestingly, the *omp*A sequences from both sub-clades were more similar to *omp*A sequences from ruminant and koala strains than to those of other pig strains; this lack of host-specificity for certain strains is a known phenomenon for *C. pecorum* (Jelocnik, 2019). However, *ompA* is no longer considered an appropriate gene to predict whole-genome phylogenies because it frequently undergoes

421 homologous recombination, as described for *C. pecorum* (Yousef Mohamad et al., 2008). The
422 phylogenetic incongruity between *ompA* genotyping and housekeeping genes including genes
423 used for MLST analysis (Jelocnik et al., 2013) could also be confirmed in this study. We
424 identified two genetically distinct novel MLST sequence types, 301 and 302, which clustered
425 closely together into a well-supported clade. ST 201 was the most similar sequence type, and
426 was detected in a fecal sample from Australian cattle. The remaining STs from livestock
427 animals as well as koalas and wild ruminants from various geographical locations formed
428 diverse separate sub-clades within a larger clade.
429 Of particular interest was the comparison with two other molecular studies, one concerning
430 MLST analysis of *C. pecorum* strains identified in Swiss alpine ibex and red deer (Jelocnik et
431 al., 2015b), and the other concerning Austrian pigs (Mohamad et al., 2014). All of these strains
432 clustered with the second larger clade and not with the sequences obtained from Swiss pigs
433 (Mohamad et al., 2014). These findings support the hypothesis that there are no host-specific
434 *C. pecorum* strains. Therefore, phylogenetic analyses conducted in this and previous studies
435 (Sait et al., 2014; Jelocnik et al., 2015b) are indicative for a global distribution of *C. pecorum*
436 strains as has been suggested for *C. suis* (Seth-Smith et al., 2017). Taken together, genotyping
437 and subsequent phylogenetic analysis revealed seven novel *C. pecorum* genotypes clustering
438 in distinct clades within global *C. pecorum* populations.
439
440
441 **Conclusion**
442 In summary, this study provides an insight into chlamydial load dynamics during the course of
443 the fattening period with a special focus on *C. pecorum* in Swiss fattening pig farms. Apart
444 from confirming previous findings such as decreasing positivity rates for *C. suis* and increasing
445 rates for *C. pecorum* between the first and second sampling timepoint (Hoffmann et al., 2015),

we could show that *C. suis* bacterial loads decreased, especially if prophylactic antibiotics were used. In contrast, the bacterial load of *C. pecorum* significantly increased, suggesting that *C. pecorum* strongly contributed to the allover *Chlamydia* load at the end of the fattening period but not at the beginning. Furthermore, we confirmed that outdoor access and contact to ruminants were risk factors for *C. pecorum* infection, which indicated that pigs are infected with ruminant *C. pecorum* strains. Genotyping using *ompA* sequencing and MLST as well as subsequent phylogenetic analyses clustered the Swiss porcine *C. pecorum* into a distinct grouping within one of the two major phylogenetic clades, unrelated to strains detected in Swiss wild ruminants, or other European *C. pecorum* strains obtained from pigs. Therefore, in order to confirm the hypothesis that domestic ruminants are responsible for infection with *C. pecorum* in pigs, strains obtained from Swiss livestock other than pigs have to be investigated in the future.

Acknowledgments

The laboratory work was partly performed using the logistics of the Center for Clinical Studies at the Vetsuisse Faculty of the University of Zurich. We would like to thank Theresa Pesch, Barbara Prähauser and Carmen Kaiser for technical support and Christiane Schnee as well as Jasmin Kuratli for helpful discussions in establishing the species-specific real-time PCRs and statistical analysis, respectively.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declarations of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Mächler, M., Bolker, B.M., 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R J. 9, 378–400. doi:10.32614/rj-2017-066

De Puysseleyr, K., De Puysseleyr, L., Dhondt, H., Geens, T., Braeckman, L., Morré, S.A., Cox, E., Vanrompay, D., 2014. Evaluation of the presence and zoonotic transmission of Chlamydia suis in a pig slaughterhouse. BMC Infect. Dis. 14, 560. doi:10.1186/s12879-014-0560-x

De Puysseleyr, L., De Puysseleyr, K., Braeckman, L., Morré, S.A., Cox, E., Vanrompay, D., 2017. Assessment of Chlamydia suis Infection in Pig Farmers. Transbound. Emerg. Dis. 64, 826–833. doi:10.1111/tbed.12446

Dean, D., Rothschild, J., Ruettger, A., Kandel, R.P., Sachse, K., 2013. Zoonotic Chlamydiaceae Species Associated with Trachoma, Nepal. Emerg. Infect. Dis. 19, 1948–1955. doi:10.3201/eid1912.130656

Dugan, J., Rockey, D.D., Jones, L., Andersen, A.A., 2004. Tetracycline Resistance in Chlamydia suis Mediated by Genomic Islands Inserted into the Chlamydial inv-Like Gene. Antimicrob. Agents Chemother. 48, 3989–3995. doi:10.1128/AAC.48.10.3989-3995.2004

Ehricht, R., Slickers, P., Goellner, S., Hotzel, H., Sachse, K., 2006. Optimized DNA microarray assay allows detection and genotyping of single PCR-amplifiable target

copies. Mol. Cell. Probes 20, 60–63. doi:10.1016/j.mcp.2005.09.003

Englund, S., Hård af Segerstad, C., Arnlund, F., Westergren, E., Jacobson, M., 2012. The occurrence of Chlamydia spp. in pigs with and without clinical disease. BMC Vet. Res. 8, 9. doi:10.1186/1746-6148-8-9

Ghasemian, E., Inic-Kanada, A., Collingro, A., Tagini, F., Stein, E., Alchalabi, H., Schuerer, N., Keše, D., Babiker, B.E., Borel, N., Greub, G., Barisani-Asenbauer, T., 2018. Detection of chlamydiaceae and chlamydia-like organisms on the ocular surface of children and adults from a trachoma-endemic region. Sci. Rep. 8, 1–16. doi:10.1038/s41598-018-23887-1

Giannitti, F., Anderson, M., Miller, M., Rowe, J., Sverlow, K., Vasquez, M., Cantón, G., 2016. Chlamydia pecorum: fetal and placental lesions in sporadic caprine abortion. J. Vet. Diagn. Invest. 28, 184–9. doi:10.1177/1040638715625729

Guscetti, F., Schiller, I., Sydler, T., Heinen, E., Pospischil, A., 2009. Experimental enteric infection of gnotobiotic piglets with Chlamydia suis strain S45. Vet. Microbiol. 135, 157–168. doi:10.1016/j.vetmic.2008.09.038

Hadfield, J.D., 2010. MCMC methods for multi-response generalized linear mixed models: The MCMCglmm R package. J. Stat. Softw. 33, 1–22. doi:10.18637/jss.v033.i02

Hoffmann, K., Schott, F., Donati, M., Di Francesco, A., Hässig, M., Wanninger, S., Sidler, X., Borel, N., 2015. Prevalence of Chlamydial Infections in Fattening Pigs and Their Influencing Factors. PLoS One 10, e0143576. doi:10.1371/journal.pone.0143576

Holzwarth, N., Pospischil, A., Marreros, N., Ryser-Degiorgis, M.-P., Mavrot, F., Frey, J., Thoma, R., Borel, N., 2011. Alpine ibex (Capra i. ibex) is not a reservoir for chlamydial infections of domestic ruminants and humans. Eur. J. Wildl. Res. 57, 233–240. doi:10.1007/s10344-010-0416-7

Jelocnik, M., 2019. Chlamydiae from Down Under: The Curious Cases of Chlamydial

520 Infections in Australia. Microorganisms 7, 602. doi:10.3390/microorganisms7120602
521 Jelocnik, M., Bachmann, N.L., Kaltenboeck, B., Waugh, C., Woolford, L., Speight, K.N.,
522 Gillett, A., Higgins, D.P., Flanagan, C., Myers, G.S.A., Timms, P., Polkinghorne, A.,
523 2015a. Genetic diversity in the plasticity zone and the presence of the chlamydial
524 plasmid differentiates Chlamydia pecorum strains from pigs, sheep, cattle, and koalas.
525 BMC Genomics 16, 893. doi:10.1186/s12864-015-2053-8
526 Jelocnik, M., Frentiu, F.D., Timms, P., Polkinghorne, A., 2013. Multilocus Sequence
527 Analysis Provides Insights into Molecular Epidemiology of Chlamydia pecorum
528 Infections in Australian Sheep, Cattle, and Koalas. doi:10.1128/JCM.00992-13
529 Jelocnik, M., Polkinghorne, A., Pannekoek, Y., 2019. Multilocus Sequence Typing (MLST)
530 of Chlamydiales, in: Methods in Molecular Biology. Humana Press Inc., pp. 69–86.
531 doi:10.1007/978-1-4939-9694-0_7
532 Jelocnik, M., Self, R., Timms, P., Borel, N., Polkinghorne, A., 2015b. Novel Sequence Types
533 of Chlamydia pecorum Infect Free-Ranging Alpine Ibex (Capra ibex) and Red Deer
534 (Cervus elaphus) in Switzerland. J. Wildl. Dis. 51, 479. doi:10.7589/2014-08-220
535 Jensen, M.L., Thymann, T., Cilieborg, M.S., Lykke, M., Mølbak, L., Jensen, B.B., Schmidt,
536 M., Kelly, D., Mulder, I., Burrin, D.G., Sangild, P.T., 2014. Antibiotics modulate
537 intestinal immunity and prevent necrotizing enterocolitis in preterm neonatal piglets.
538 Am. J. Physiol. Liver Physiol. 306, G59–G71. doi:10.1152/ajpgi.00213.2013
539 Joseph, S.J., Marti, H., Didelot, X., Read, T.D., Dean, D., 2016. Tetracycline Selective
540 Pressure and Homologous Recombination Shape the Evolution of Chlamydia suis : A
541 Recently Identified Zoonotic Pathogen. Genome Biol. Evol. 8, 2613–2623.
542 doi:10.1093/gbe/evw182
543 Kaltenboeck, B., Storz, J., 1992. Biological properties and genetic analysis of the ompA locus
544 in chlamydiae isolated from swine. Am. J. Vet. Res. 53, 1482–7.

Li, J., Guo, W., Kaltenboeck, B., Sachse, K., Yang, Y., Lu, G., Zhang, J., Luan, L., You, J., Huang, K., Qiu, H., Wang, Y., Li, M., Yang, Z., Wang, C., 2016. Chlamydia pecorum is the endemic intestinal species in cattle while C . gallinacea, C. psittaci and C. pneumoniae associate with sporadic systemic infection. Vet. Microbiol. 193, 93–99. doi:10.1016/j.vetmic.2016.08.008

Li, M., Jelocnik, M., Yang, F., Gong, J., Kaltenboeck, B., Polkinghorne, A., Feng, Z., Pannekoek, Y., Borel, N., Song, C., Jiang, P., Li, J., Zhang, J., Wang, Y., Wang, J., Zhou, X., Wang, C., 2017. Asymptomatic infections with highly polymorphic Chlamydia suis are ubiquitous in pigs. BMC Vet. Res. 13, 370. doi:10.1186/s12917-017-1295-x

Mohamad, K.Y., Kaltenboeck, B., Rahman, K.S., Magnino, S., Sachse, K., Rodolakis, A., 2014. Host adaptation of Chlamydia pecorum towards low virulence evident in co-evolution of the ompA, incA, and ORF663 loci. PLoS One 9, 103615. doi:10.1371/journal.pone.0103615

Pannekoek, Y., Morelli, G., Kusecek, B., Morré, S.A., Ossewaarde, J.M., Langerak, A.A., Van Der Ende, A., 2008. Multi locus sequence typing of Chlamydiales: Clonal groupings within the obligate intracellular bacteria Chlamydia trachomatis. BMC Microbiol. 8, 42. doi:10.1186/1471-2180-8-42

Pantchev, A., Sting, R., Bauerfeind, R., Tyczka, J., Sachse, K., 2010. Detection of all Chlamydophila and Chlamydia spp. of veterinary interest using species-specific real-time PCR assays. Comp. Immunol. Microbiol. Infect. Dis. 33, 473–84. doi:10.1016/j.cimid.2009.08.002

Pillonel, T., Bertelli, C., Aeby, S., de Barsy, M., Jacquier, N., Kebbi-Beghdadi, C., Mueller, L., Vouga, M., Greub, G., 2019. Sequencing the Obligate Intracellular Rhabdochlamydia helvetica within Its Tick Host Ixodes ricinus to Investigate Their

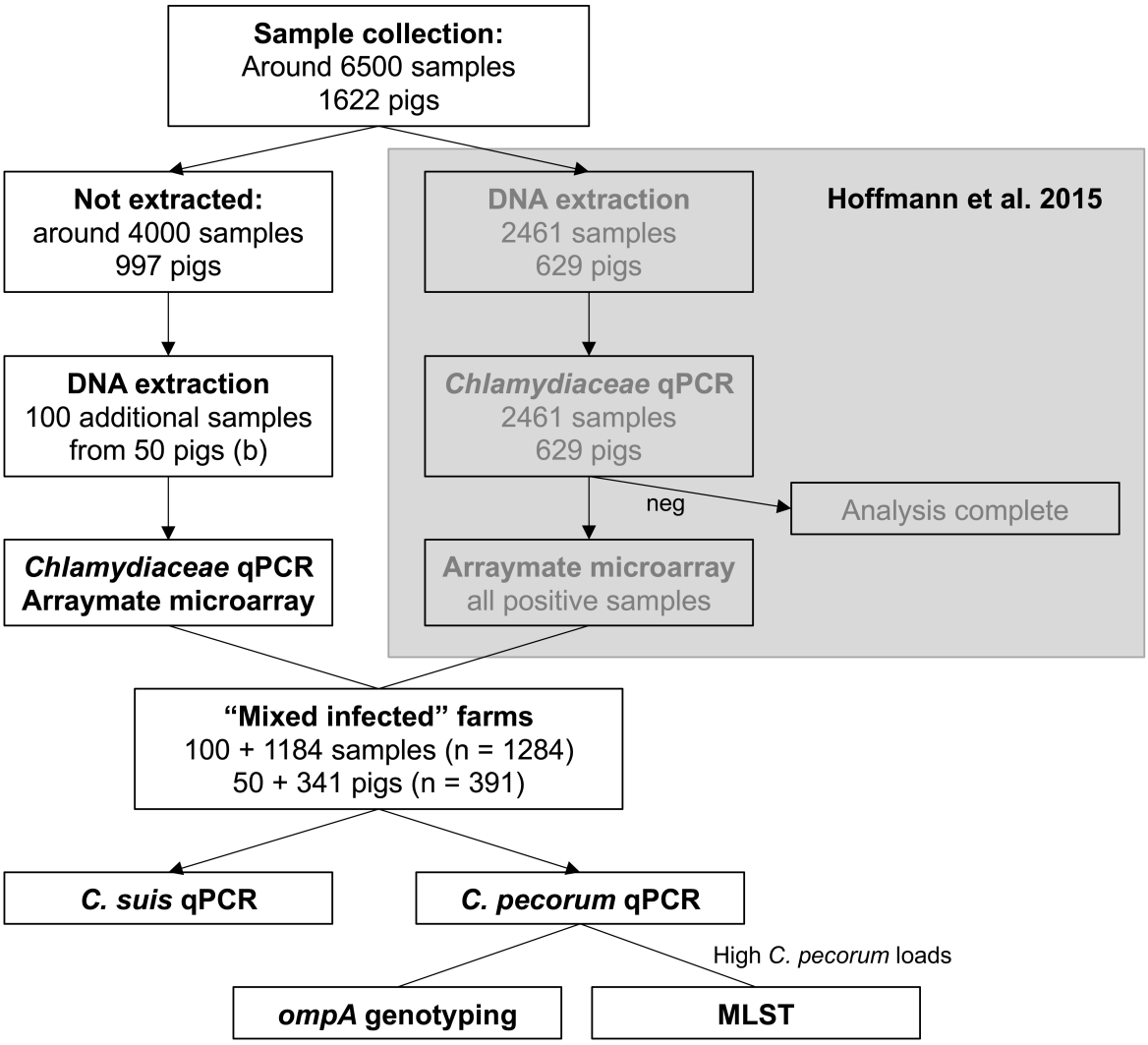
570 Symbiotic Relationship. Genome Biol. Evol. 11, 1334–1344. doi:10.1093/gbe/evz072
571 Reinhold, P., Sachse, K., Kaltenboeck, B., 2011. Chlamydiaceae in cattle: Commensals,
572 trigger organisms, or pathogens? Vet. J. 189, 257–267. doi:10.1016/j.tvjl.2010.09.003
573 Sachse, K., Borel, N., 2020. Recent Advances in Epidemiology, Pathology and Immunology
574 of Veterinary Chlamydiae, in: Tan, M., Hegeman, J.H., Sütterlin, C. (Eds.), Chlamydia
575 Biology: From Genome to Disease. Caister Academic Press, Norfolk, pp. 403–428.
576 doi:10.21775/9781912530281.17
577 Sait, M., Livingstone, M., Clark, E.M., Wheelhouse, N., Spalding, L., Markey, B., Magnino,
578 S., Lainson, F.A., Myers, G.S.A., Longbottom, D., 2014. Genome sequencing and
579 comparative analysis of three Chlamydia pecorum strains associated with different
580 pathogenic outcomes. BMC Genomics 15. doi:10.1186/1471-2164-15-23
581 Schautteet, K., Beeckman, D.S.A., Delava, P., Vanrompay, D., 2010. Possible pathogenic
582 interplay between Chlamydia suis, Chlamydophila abortus and PCV-2 on a pig
583 production farm. Vet. Rec. 166, 329–333. doi:10.1136/vr.b4714
584 Schautteet, K., Vanrompay, D., 2011. Chlamydiaceae infections in pig. Vet. Res. 42, 29.
585 doi:10.1186/1297-9716-42-29
586 Schnee, C., Sachse, K., 2015. DNA microarray-based detection of multiple pathogens:
587 Mycoplasma spp. and Chlamydia spp. Methods Mol. Biol. 1247, 193–208.
588 doi:10.1007/978-1-4939-2004-4_15
589 Seth-Smith, H.M.B., Wanninger, S., Bachmann, N., Marti, H., Qi, W., Donati, M., di
590 Francesco, A., Polkinghorne, A., Borel, N., 2017. The Chlamydia suis Genome Exhibits
591 High Levels of Diversity, Plasticity, and Mobile Antibiotic Resistance: Comparative
592 Genomics of a Recent Livestock Cohort Shows Influence of Treatment Regimes.
593 Genome Biol. Evol. 9, 750–760. doi:10.1093/gbe/evx043
594 Unterweger, C., Schwarz, L., Jelocnik, M., Borel, N., Brunthaler, R., Inic-Kanada, A., Marti,

H., 2020. Isolation of Tetracycline-Resistant Chlamydia suis from a Pig Herd Affected by Reproductive Disorders and Conjunctivitis. Antibiotics 9, 187. doi:10.3390/antibiotics9040187

Wahdan, A., Rohner, L., Marti, H., Bacciarini, L.N., Menegatti, C., Di Francesco, A., Borel, N., 2020. Prevalence of Chlamydiaceae and Tetracycline Resistance Genes in Wild Boars of Central Europe. J. Wildl. Dis. 2019-11–275. doi:10.7589/2019-11-275

Yousef Mohamad, K., Roche, S.M., Myers, G., Bavoil, P.M., Laroucau, K., Magnino, S., Laurent, S., Rasschaert, D., Rodolakis, A., 2008. Preliminary phylogenetic identification of virulent Chlamydophila pecorum strains. Infect. Genet. Evol. 8, 764–771. doi:10.1016/j.meegid.2008.06.009

607 **Figure captions**



608

609 **Figure 1. Sample collection and workflow.** Shown are the different methods used in this
610 study. From a collection of approximately 6500 samples from 1622 pigs, 2461 samples
611 from 629 pigs were already processed by DNA extraction, *Chlamydiaceae* qPCR and, if
612 positive, by Arraymate microarray in a previous study (Hoffmann et al., 2015).
613 Furthermore, 100 additional samples from 50 pigs in farms with known
614 *C. suis/C. pecorum* mixed infection, collected at the end of the fattening period (sampling
615 timepoint b), were processed similarly. A total of 1284 samples from 391 pigs were then
616 analyzed by *C. suis* and *C. pecorum*-specific qPCRs to determine the bacterial load for
617 each species. From a selection of *C. pecorum* samples with high chlamydial loads, major

outer membrane *(ompA)* genotyping and multi-locus sequence typing (MLST) was performed.

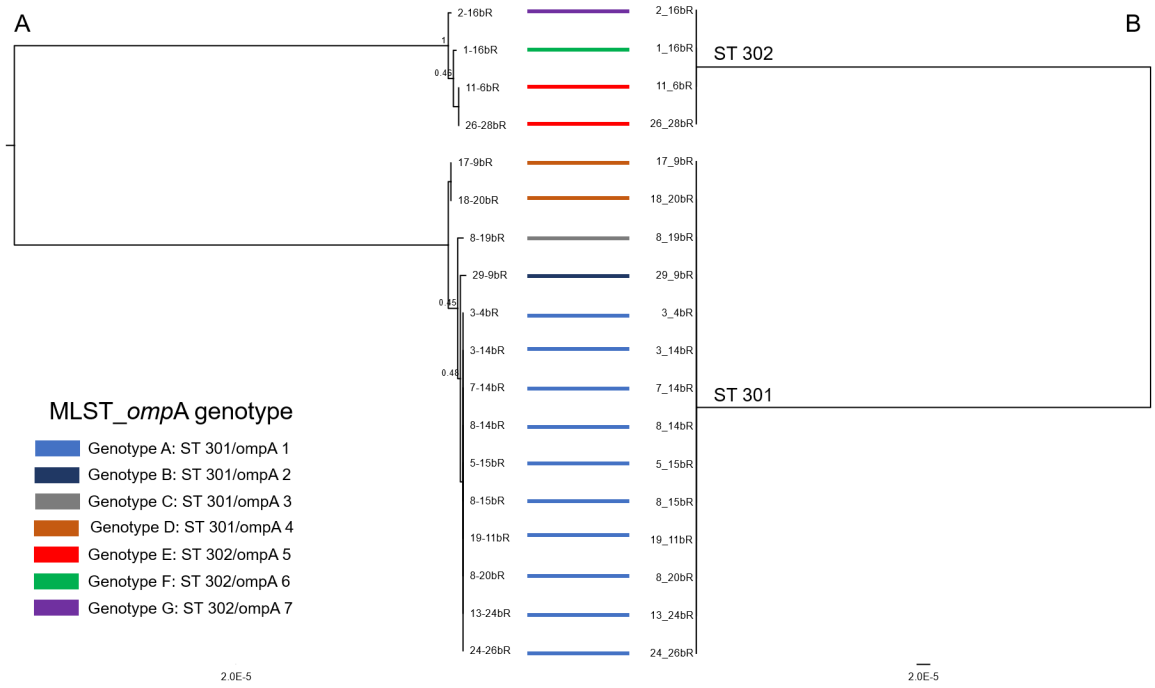
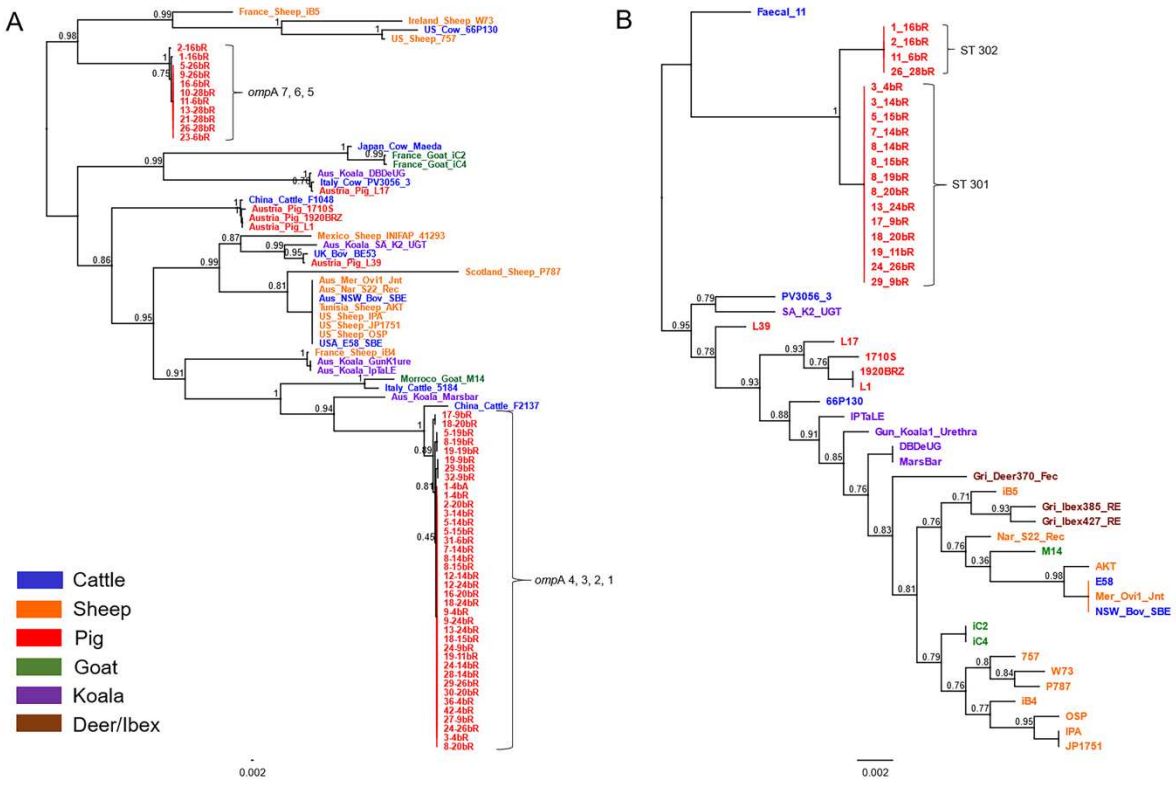


Figure 2. Tanglegram of *C. pecorum ompA* and MLST Bayesian trees. Shown is a tanglegram matching *C. pecorum* taxa on (A) *ompA* and (B) MLST mid-point rooted approximately-maximum-likelihood trees of eighteen samples from twelve different Swiss fattening pig farms. The seven resulting genotypes A-G are depicted in different colors connecting the same taxa on two trees: *ompA* genotypes are listed in the legend (bottom left), while the two MLST sequence types (ST) 301 and 302 are depicted both in the legend and in the tree.



632

633 **Figure 3. *C. pecorum ompA* and MLST phylogenetic trees of globally distributed strains.**

634 Depicted are the (A) *ompA* and (B) MLST mid-point rooted approximately-maximum-

635 likelihood phylogenetic trees of the Swiss fattening pigs (red, bold letters) compared to strains

636 from different countries and host species. Support values are displayed on tree nodes. Host

637 species are depicted with different colors: cattle (blue), sheep (orange), pig (red), goat (green),

638 koala (purple), deer/ibex (brown), while the geographical origin of the strain is indicated in the

639 strain names of the *ompA* tree.

640

641 **Table 1.** Occurrence of clinical signs in fattening pigs on farms 1-14

Farm	HK Farm[1]	Antibiotics[2]	Conjunctivitis		Diarrhea	
			a[3]	b	a	b
1	4	No	1/22	2/50	0/22	5/50
2	6	No	1/21	1/30	0/21	0/30
3	9	No	1/36	7/36	1/36	1/36
4	11	No	0/21	0/20	0/21	0/20
5	14	No	9/21	8/28	3/21	0/28
6	15	TSS	2/20	12/20	6/20	0/20
7	16	No	1/25	10/25	0/25	0/25
8	19	No	0/24	5/20	0/24	0/20
9	20	CTS	2/21	14/30	3/21	0/30
10	22	CTS	3/30	25/30	0/30	0/30
11	24	No	1/20	18/20	1/20	0/20
12	25	No	13/20	17/20	0/20	0/20
13	26	TSS	13/20	24/30	1/20	0/30
14	28	CTS	3/22	2/20	0/22	0/20

642 [1]Farm numbers of the Hoffmann et al. (2015) study

643 [2]Prophylactic antibiotics used at the beginning of the fattening period (timepoint a): No
644 antibiotics (No), Trimethoprim, sulfadimidine, sulfathiazole (TSS), chlortetracycline, tylosin,
645 sulfadimidine (CTS)

646 [3]Timepoint a = sampling at the beginning of the fattening period; b = sampling at the end of
647 the fattening period

648

649

650 **Table 2.** Positivity rate of *C. pecorum* on farm-level

Farm	HK Farm[1]	Tested (a/b)[2]	Positive a+b[3]	Positive a	Positive b
1	4	49 (22/47)	-	-	38
2	6	31 (21/30)	-	-	18
3	9	36 (36/36)	1 (36)	2	11
4	11	21 (21/20)	-	-	6
5	14	31 (21/28)	-	-	15
6	15	20 (20/20)	-	-	5
7	16	25 (25/20)	-	-	12
8	19	24 (24/20)	-	-	2
9	20	31 (21/30)	-	-	17
10	22	30 (30/20)	-	-	-
11	24	20 (20/20)	-	-	7
12	25	20 (20/20)	-	-	12
13	26	30 (20/30)	-	-	26
14	28	22 (22/20)	-	-	9

651 [1]Farm numbers of the Hoffmann et al. (2015) study

652 [2]Number of animals tested at the beginning (a) and end (b) of the fattening period

653 [3]Values in parentheses represent the number of animals investigated at both sampling
654 timepoints

655

Table 3. Bacterial load changes from the first (a) to the second (b) sampling timepoint per qPCR

qPCR	Animals tested (n)[1]	a (copies/swab)[2]	b (copies/swab)[2]	Load changes[3]	Statistical significance (p-values)[4]
Chlamydiaceae	294	$3x10^5$ ($4x10^3$, $5x10^5$)[5]	$7x10^4$ ($5x10^4$, $9x10^4$)	↓ (↑, ↓)	$1.4\ x10^{-4}$ ($2.3x\ 10^{-6}$, $2.5x\ 10^{-11}$)
C. suis	288	$3x10^5$ ($2x10^3$, $5x10^5$)	$2x10^4$ ($3x10^4$, $2x10^4$)	↓ (↑, ↓)	$1.4\ x10^{-4}$ ($2.3x\ 10^{-6}$, $2.5x\ 10^{-11}$)
C. pecorum	290	$1x10^2$ ($1x10^2$, 0)	$4x10^4$ ($8x10^2$, $5x10^4$)	↑ (↑, ↑)	$9.5\ x10^{-10}$

[1]Number of animals tested per PCR. DNA availability was the limiting factor, which prevented all 294 animals from being tested with the species-specific qPCRs.

[2]The bacterial load is expressed as the predicted number of chlamydial genome copies per swab

[3]Relative changes of the bacterial loads between the first (a) and second (b) sampling timepoint: total (conjunctival, rectal)

[4]p-values comparing genome copies per swab between sampling timepoints: total (conjunctival, rectal)

[5]Average number of genome copies per swab: total (conjunctival, rectal)

Table 4. List of combined MLST/*ompA* *C. pecorum* genotypes

Combined MLST/*ompA* genotype	***ompA* genotype**	**MLST (ST)[1]**	**Samples[2]**
A	1	301	3-4bR, 19-11bR, 3-14bR, 7-14bR, 8-14bR, 5-15bR, 8-15bR, 8-20bR, 13-24bR, 24-26bR
B	2	301	29-9bR
C	3	301	8-19bR
D	4	301	17-9bR, 18-20bR
E	5	302	11-6bR, 26-28bR
F	6	302	1-16bR
G	7	302	2-16bR

[1]MLST Sequence Types (ST)

[2]Samples are depicted as "Animal Number, Farm Number, sampling timepoint (a = first sampling, b = second sampling) and type (R = rectal)." For example, sample 3-4bR is from animal no. 3 of farm no. 4 (Hoffmann et al. 2015). It was a rectal swab from the second timepoint.